and subunit dynamics of these transporters, it will be necessary to monitor the conformational changes in real time and at the level of individual subunits. Single-molecule techniques seem therefore ideal to answer the open questions regarding the glutamate transport family. We have probed the transport of aspartate through individual trimers of the archeal Na+ coupled aspartate transporter GltPh with single-molecule fluorescence and we were able to monitor the conformational changes associated with substrate translocation. These data allow us to refine our view of the different conformations involved in transport.

1972-Plat
Directly Counting Nucleoporins Inside the Yeast Nuclear Pore Complex
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The nuclear pore complex (NPC) is one of the largest supramolecular structures in eukaryotic cells. Its octagonal ring-scaffold perforates the nuclear envelope and houses a molecular sortase that regulates nucleocytoplasmic transport. It is composed of ~30 different nucleoporins (Nups), estimated at 8, 16 or 32 copies per NPC, although this has not been confirmed due to the inherent difficulty of counting protein within supramolecular complexes. Here we used single-molecule SPEED microscopy to directly count the copy-number of twenty-four different Nups within the NPC of live yeast, and found significant deviations from previous estimates. Each NPC contained a maximum of 16 copies of Nsp1 and Nic96, rather than 32 as previously estimated; 16 copies of thirteen additional Nups; 8 copies of four Nups; and 10-15 copies of five Nups. This in situ molecular-counting technology can help resolve the architecture of NPCs and other supramolecular structures in living cells.

1973-Plat
Measuring the Pore-Size and Dynamics of the MscL Mechanosensitive Channel using smFRET
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Mechanosensitive ion channels are membrane-bound proteins that let solutes (and water) flow in and out of the cell in response to membrane deformation. They are involved in sensory modalities, like touch, sound, fluid balance, and blood pressure. The mechanosensitive channel of large conductance (MscL) is to date the best studied mechanosensitive channel and serves as a model for channel mechanosensitivity. MscL has been crystallized in the closed form, but not in the open form. Some fundamental questions remain to date not fully answered including - what is the diameter of the open channel pore? Previously, several reports described open channel structure of MscL that was obtained by using either EPR or FRET spectroscopy on large ensembles of MscL channels. Although these studies provided good estimates of the open MscL pore, the size of the pore of a fully open channel still remains to be determined. Here we report a study describing both the open and closed states of MscL using single molecule fluorescence energy transfer (smFRET). We measured the distance changes of a number of residues from the three MscL alpha-helices TM1, TM2 and CP , i.e. I25, A27, and M42 on TM1; Y75 and I96 on TM2 and A110 and V120 on CP. Since we measured two or more sites on each alpha helix, rotational tilting angles, as well as the translational movements of the helices could be obtained. From these measurements, a structure of the protein in the open state was determined, using the crystal structure of the closed channel as a reference. From the obtained open structure we could determine the pore size of the fully open channel. To understand the channel mechanosensitivity, we investigated the dynamics of the conformational change of the channel.

Workshop 3: Biophysics Inside the Cell

1974-Wkshp
Understanding How Chromatin Structure and Dynamics affect Transcription In Vivo
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Nucleosomes, which are the basic packaging units of chromatin, are stably positioned in promoters upstream of most stress-inducible genes. These promoter nucleosomes are generally thought to prevent transcription factors from accessing their target sites on the DNA. However, nucleosomes are not static, but can be partially destabilized through spontaneous unwrapping of DNA from the histone octamer or can be removed altogether. Moreover, they can participate in transcriptional activation by providing a sliding wheel for chromatin remodeling complexes. To shed light on how chromatin structure and dynamics affect transcriptional activation, we perturb positioning and stability of promoter nucleosomes and measure the downstream effect on transcriptional dynamics in single budding yeast cells. Combining results from time-lapse microscopy and absolute nucleosome occupancy measurement, we build a quantitative kinetic model for eukaryotic promoter transitions. This work provides insights into the dynamic regulatory code of the genome.

1975-Wkshp
Imaging and Sequencing Single Molecules in Individual Cells
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Chem/Chem Biol, Harvard University, Cambridge, MA, USA.
Recent advances in single-molecule imaging in living cells allow quantitative and system-wide descriptions of gene expression and regulation with single molecule sensitivity. It was found that low probability events of single molecules can have important biological consequences, such as the change of a cellular phenotype. This has everything to do with the fact that DNA are single molecules in individual cells. Meanwhile, recent advances in high throughput DNA sequencing and whole genome and transcriptome amplification have allowed sequencing the genome and transcriptome of a single human cell. The combination of single-molecule imaging and sequencing in single cells offers exciting possibilities for understanding dynamics in living systems.

1976-Wkshp
Stochastic Simulations of Cellular Processes in Bacteria
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Stochastic expression of genes produces heterogeneity in clonal populations of bacteria grown under identical conditions. We analyze the stochastic reaction-diffusion dynamics of selected biochemical pathways in *Escherichia coli* to show how individual cells vary expression of a set of genes in response to an environmental signal. The whole cells simulated under *in vivo* conditions include ribosomes, DNA, and large protein complexes, which take up 30-50% of the cell volume and are placed according to data from cryoelectron tomography and proteomics. using GPU processors, we simulate the dynamics for an entire cell cycle and compare the mRNA/protein distributions to those observed in single molecule experiments. We show how such distributions can be used to derive additional kinetic parameters and integrate effects of cell-to-cell variations into flux balance analysis of genome scale models of metabolic networks. The distribution of growth rates calculated for a colony of bacteria are analyzed and correlated to changes in fluxes through the metabolic network.
With the availability of high-performance computing, simulations are poised to allow integration of data from structural, single-molecule, and biochemical studies into coherent computational models of cells and cellular processes. Here the calculations are performed with our Lattice Microbes software. Animation of reaction trajectories involving millions of particles is facilitated using a plugin to the VMD visualization and analysis program.
“Noise contributions in an inducible genetic switch: A whole cell simulation study”, E. Roberts, A. Magis, J. Ortiz, W. Baumeister, and Z. Luthey-Schulten, *Plos Comput. Biol*. 7(3), e1002010 March (2011).
“Determining the stability of genetic switches: Explicitly accounting for mRNA noise”
M. Assaf, E. Roberts, and Z. Luthey- Schulten, *Phys.Rev.Lett*. 24, 248102, (2011).
“Lattice Microbes: high-performance stochastic simulation method for the reaction-diffusion master equation”, E. Roberts, J. Stone, Z. Luthey-Schulten, *J. Comp. Chem,* (2012, in press).

1977-Wkshp
Physical Aspects of Spindle Assembly
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The spindle is a complex assembly of microtubules, motors, and other associated proteins, which segregate chromosomes during cell division.
In metaphase, the spindle exists in a steady-state with a constant flux of molecules and energy continuously modifying and maintaining its architecture. While the self-organization of systems of microtubules and motors have been investigated using theory and experiments, there have been few attempts to test if the proposed theories can be used to understand the dynamics and structure of complex biological systems in vivo. Here we use polarized light microscopy, 3D time-lapse spinning disk confocal microscopy, single molecule imaging, second harmonic generation microscopy, and mechanical measurements to test the validity of continuum models of metaphase spindles. Our results show that a simple continuum model can quantitatively explain spindle structure and dynamics, demonstrate that rigorous physical theories can be used to quantitatively describe complex subcellular systems, and provides